A MODIFIED RESPIROMETER FOR STUDIES ON THE RESPIRATORY QUOTIENT OF APPLES.

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(One Text-figure.)

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In previous studies in the metabolism of apples by Trout *et al.* (1942) and Hackney (1943*a*, 1943*b*) no values for the respiratory quotient were obtained. Such values were thought to be desirable in order to indicate the type of respiration being carried on.

Methods such as those of Magness and Diehl (1924), Haller and Rose (1932) and Platenius (1942) have been used to measure the respiratory quotient of batches of more than one fruit. Besides being unsuitable for work on individual fruits these methods have the disadvantage that they usually require several days for a single reading.

The method described by the present writer has been used satisfactorily to measure the respiratory quotients of individual apples. Usually the time required for one reading has been not more than three hours.

The apparatus is shown in Fig. 1. It is essentially a differential volumeter of the type used by Fenn (1935) and modified by Duryee (1936), Thimann and Commoner (1940) and Tyler and Berg (1941).

Each of the vessels, A, B, consists of two thick-walled hemispheres with flat-ground glass flanges which can be quickly separated or sealed together with petroleum jelly. Vessel A is the respiration chamber in which the fruit is put; vessel B is the compensating chamber. The capacity of each vessel is about 500 ml.

The vessels are connected to each other by means of the graduated capillary tube, C_1, which contains a small index drop of kerosene, coloured with Sudan III. By means of 3-way stopcocks, D_1 and D_2, the atmospheres of the two vessels can be brought into contact with the index drop.

The long graduated capillary tube, C_2, contains a column of mercury the level of which is adjusted by means of a screw-clip, E, compressing the rubber tubing at the end of the capillary. The bore of the capillary tubing is about 0·8 mm. and is coarse enough to allow free movement of the index drop. The small graduations on tube C_2 are about 1 mm. apart. Each division is equivalent to a volume of 0·00213 ml. The full length of the scale is equivalent to 0·526 ml.

The two vessels are immersed in water which is vigorously agitated by the mechanical stirrer, S, set directly between them. The whole apparatus is kept in a room maintained at constant temperature.

To determine the respiratory quotient (R.Q.) of the fruit, it is necessary to measure the rates of uptake of oxygen and output of carbon dioxide. In order to measure the rate of uptake of oxygen an absorbent for carbon dioxide is introduced into the respiration chamber. Granular soda-lime was found to be most suitable. In preliminary experiments, N/10 barium hydroxide was introduced through the bottom of the sphere, as shown in Fig. 1A. It was found that the efficiency of absorption was lowered by the formation of a film of carbonate on the surface of the liquid. A concentrated solution of potassium hydroxide was tried and rejected because of the danger of its coming into contact with the fruit.

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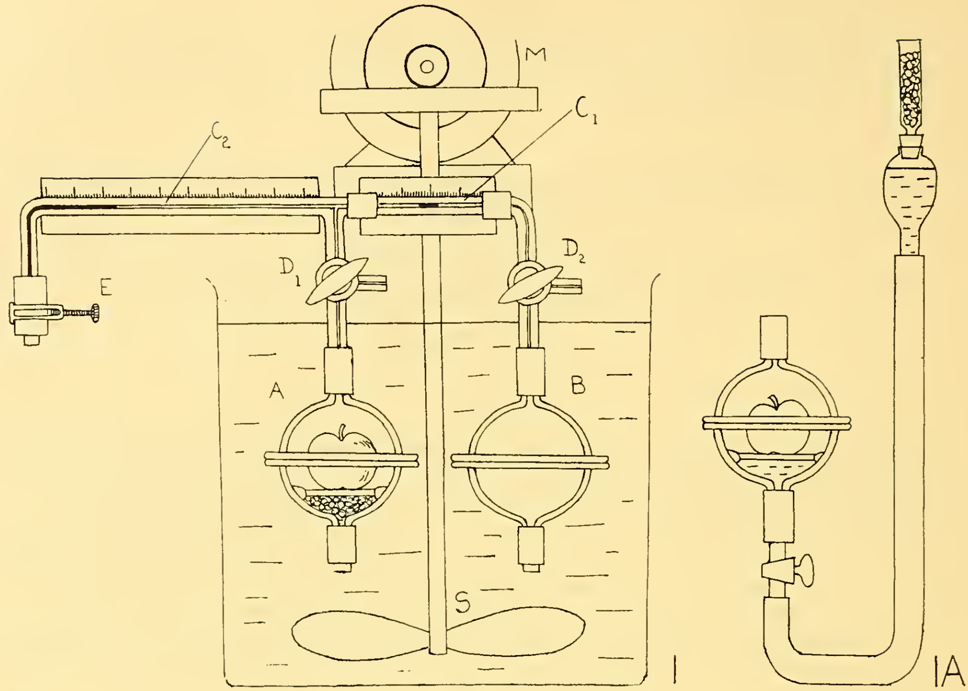

Fig. 1.

To measure the rate of oxygen uptake the following procedure is adopted:

The vessel, A, containing about 20 gm. of soda-lime, is disconnected from the tap, D_1. The two hemispheres are separated and the apple is placed on a small glass platform above the soda-lime. The hemispheres are then sealed together and connected to the apparatus. The taps are turned so that the atmosphere of each vessel is in contact with the external atmosphere. A period of at least 1¼ hours is then allowed for the apparatus to come to the temperature of the water and for equilibrium to be established between the production of carbon dioxide from the fruit and its absorption by the soda-lime.

The taps are then turned so that the atmosphere in the respiration chamber, A, is connected with the mercury column and the index drop, and the compensating chamber, B, is connected with the index drop. After 5 minutes have been allowed for further equilibration, the drop is brought level with a fixed mark on the centre of tube C_1, by moving the mercury column in tube C_2. The time and the reading of the mercury on the scale are recorded.

After a period of 10–30 minutes, according to the magnitude of the rate of uptake of oxygen, the index drop is again adjusted to its original position. The difference between the final position of the mercury column and the original reading is used to calculate the decrease in volume which has occurred.

The whole procedure is then repeated without the carbon dioxide absorbent. The respiration chamber, A, is disconnected and the fruit removed. The fruit is transferred to an empty vessel which is connected to the apparatus in place of the previous vessel, A. Changes in volume are then measured in the same way as has been described for the determination of rate of uptake of oxygen.

If any decrease occurs in the volume of the air surrounding the apple (R.Q.$<$1), the index drop in C will be displaced to the left. Any increase in the volume of the atmosphere in the experimental vessel (R.Q.$>$1) results in a movement of the drop to the right. The difference between the position of the mercury after adjustment of the index drop at the end of the period, and its original position, gives the amount of decrease or increase which has occurred.

Where observations are being made on several fruits, many readings per day can be taken by the use of a large water-bath in which extra vessels, with apples enclosed, can be brought to equilibrium before being connected to the apparatus.

The changes in volume are measured at the temperature of the water-bath and the barometric pressure at the time of commencing an observation. By correcting for temperature and pressure, the rates of oxygen uptake and carbon dioxide production can be expressed in the desired units. In this paper they are expressed in mg./10Kg./hr.

The method of calculation is as follows:

Temperature of water-bath = t°C.
Barometric pressure = p mm.

With CO_2 absorbent.

Period of observation = m min.
Decrease in volume of air surrounding fruit $\equiv$ X divisions on scale C_2
$\equiv X \times C$ c.c. at t°C. and p mm.

(Where C is the volumetric equivalent for 1 division on the scale, C_2.)

$$\therefore \text{Volume of oxygen consumed in 1 hour} = \frac{C \times X \times 60}{m} \text{ ml. at t°C. and p mm.}$$

Without CO_2 absorbent.

Period of observation = m_1 min.
Change in volume of air surrounding apple $\equiv \pm X_1$ divisions on scale C_1
$\equiv \pm X_1 \times C$ ml. at t°C. and p mm.

$$\left.\begin{matrix}\text{Increase}\\ \text{Decrease}\end{matrix}\right\} \text{ in volume in 1 hour} = \frac{C \times X_1 \times 60}{m_1} \text{ ml. at t°C. and p mm.}$$

$$\therefore \text{Volume of } CO_2 \text{ produced in 1 hour} = \frac{C \times X \times 60}{m} \pm \frac{C \times X_1 \times 60}{m_1} \text{ ml. at t°C. and p mm.}$$

$$\therefore \text{R.Q. of fruit} = \frac{\dfrac{C \times X \times 60}{m} \pm \dfrac{C \times X_1 \times 60}{m}}{\dfrac{C \times X \times 60}{m}}$$

Conversion of rates of oxygen uptake and carbon dioxide production from ml./hr. at t°C. and p mm. to mg./10Kg./hr.

$$\text{Rate of oxygen uptake} = \frac{C \times X \times 60}{m} \times \frac{273}{273 + t} \times \frac{p}{760} \times \frac{32}{22{\cdot}4} \times \frac{10^4}{w} \text{ mg./10Kg./hr.}$$

Rate of carbon dioxide production =

$$\left(\frac{C \times X \times 60}{m} + \frac{C \times X_1 \times 60}{m_1}\right) \times \frac{273}{273 + t} \times \frac{p}{760} \times \frac{44}{22{\cdot}4} \times \frac{10^4}{w} \text{ mg./10Kg./hr.}$$

(where 'w' is the weight of the fruit).

To test the efficiency of the method, comparisons were made between values of respiration rate obtained by this method and those obtained by the Pettenkofer method (Trout *et al.*, 1942) for apples whose respiration rates were known to be steady. Reasonably good agreement was obtained. Typical results are shown in Table 1.

TABLE 1.

Respiration Rate ($mg.CO_2$/10Kg./hr.).

Pettenkofer	155	154	98	96	91	115
Respirometer	147	163	97	98	105	123

As far as is known, this type of volumeter has not previously been used for measuring the respiration rate or respiratory quotient of apples. Most methods, such as those used by Magness and Diehl (1924), Haller and Rose (1932) and Platenius (1942) involve more elaborate apparatus and require longer periods of time for readings.

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The advantages of the present method are:

(1). It is independent of external temperature and pressure changes after the taps are closed to the atmosphere.

(2). Owing to the shortness of the time required for the observation, there is no appreciable alteration either in the pressure or in the composition of the atmosphere surrounding the fruit while the observation is being made.

(3). The apparatus is easily manipulated.

(4). The short duration of the readings enables more accurate information to be gained where rates of carbon dioxide production and oxygen consumption are changing rapidly.

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